pointed out where within the specification this language is found and requested that Applicants point out the page and line numbers where the language that supports the arguments can be found.

The specification, at page 14, lines 13-22, states:

> **When a quantified level of *CYP24* falls outside of a given confidence interval for a normal level of *CYP24*, the difference between the two levels is said to be "statistically significant."** If a test value falls outside of a given confidence interval for a normal level of *CYP24*, it is possible to calculate the probability that the test value is truly abnormal and does not just represent a normal deviation from the average. In the methods of this invention, a difference between a test sample and a control can be termed "statistically significant" when the probability of the test sample being abnormal can be any of a number of values, including 0.15, 0.1, 0.05, and 0.01. Numerous sources teach how to assess statistical significance, such as Freund, J.E. (1988) Modern elementary statistics, Prentice-Hall. [emphasis added]

A "statistically significant difference" is thus expressly defined. The phrase reasonably apprise[s] those skilled in the art both of the utilization and scope of the invention, and is as precise as the subject matter permits. The rejection of claims 15 and 30 under 35 U.S.C. §112, second paragraph, for the use of the phrase "statistically significant difference" is therefore improper and should be withdrawn.

35 U.S.C. §112, First Paragraph.

Claims 18-32 were rejected under 35 U.S.C. §112, first paragraph, as allegedly not enabled. In particular, the Examiner alleged that "there is no link evidenced within the specification between high levels of CYP24 and survival expectancy." Applicants respectfully traverse.

The specification, at page 17, line 27 through page 20, line 5 states:

> ***CYP24* encodes vitamin D 24 hydroxylase, an enzyme that catalyzes degradation of the active form of vitamin D, 1,25-dihydroxy-D3** (*for reviews, see* Walters (1992) *Endocrine Reviews* 13: 719-764; Jones *et al.* (1998) *Amer. Physiol. Soc.* 78: 1193-1231). Vitamin D is a secosteroid hormone that plays a major role in the regulation of calcium and bone metabolism. However, vitamin D receptors (*VDR*) have also been found in many other so-called "non-classical" tissues not involved in mineral metabolism, including the breast (Berger *et al.* (1987) *Cancer Res.* 47: 6793-6799; Buras *et al.* (1994) *Breast Cancer Res. and Treatment* 31: 191-202), indicating a role for vitamin D in these tissues also. Levels of 1,25-dihydroxy-D3 and ligand bound receptor appear to be very tightly controlled

in cells by a feedback mechanism. Binding of the hormone to the *VDR* results in activation of *CYP24* transcription to initiate degradation of 1,25-dihydroxy-D3 and inhibition of *CYP1*, the enzyme required for synthesis of 1,25-dihydroxy-D3. **In fact, transcription of *CYP24* is so closely coupled to *VDR* levels and activity that activation of transcription from a *CYP24* promoter-reporter construct is used as an assay for *VDR* activity** (Arbour *et al.* (1998) *Anal. Biochem.* 255: 148-154). Thus, without being bound to this theory, we believe the role of *CYP24* in cells is to limit the biological activity of the vitamin D system.

In the "non-classical" tissues such as breast, vitamin D promotes growth inhibition by directing cells towards differentiation and cessation of proliferation. Breast cancer cells respond to the antiproliferative effects of vitamin D both *in vivo* and *in vitro* (Eisman *et al.* (1989) *Cancer Res.* 47: 21-25). **Breast cancer cell lines generally arrest in the G0/G1 stage of the cell cycle in response to vitamin D, and the MCF-7 breast cancer cell line can be induced to enter apoptosis** (Elstner *et al.* (1995) *Cancer Res.* 55: 2822-2830; Love-Schimenti *et al.* (1996) *Cancer Res.* 56: 2789-2794; Simboli-Campbell *et al.* (1997) *Breast Cancer Res. and Treatment,* 42: 31-41). **Administration of vitamin D to rodents reduces progression of tumor xenographs** (Eisman *et al.* (1989) *Cancer Res.* 47: 21-25; Colston *et al.* (1989) *Lancet*, 188-191).

These growth modulatory properties of vitamin D support the present belief that disruption of the vitamin D system is likely to contribute to neoplasia. This suggestion is further supported by the observation that patients with receptor negative tumors have a poorer prognosis and by epidemiological studies that have established that exposure to sunlight and risk of breast and colon cancer (Gorham *et al.* (1989) *Can. J. Public Health* 80: 96-100; Gorham *et al.* (1990) *Int. J. Epidemiol.* 19: 820-824; Garland *et al.* (1990) *Preventive Medicine* 19: 614-622) are inversely correlated.

Thus, the present hypothesized oncogenic role of *CYP24* derives from its function to reduce levels of 1,25-dihydroxyvitamin-D3 and so modulate the biological effects of ligand bound *VDR*. This hypothesis is supported by the observation that the antiproliferative activity of vitamin D *in vitro* is enhanced in the presence of hydroxylase inhibitors (Reinhardt and Horst (1989) *Arch. Biochem. Biophys.* 272: 459-465; Zhao *et al.* (1996) *J. Steroid. Biochem. Mol. Biol.* 57: 197-202). Thus, without being bound by a theory, the present invention is predicated, in part, on the recognition that amplification of *CYP24* abrogates vitamin D mediated growth control by up-regulation of vitamin D degradation in cells, since ligand bound *VDR* will bind to and initiate transcription from an increased number of *CYP24* gene copies. [emphasis added]

The specification thus expressly provides a clear link between CYP24 expression level and cancer survival expectancy.

Moreover, the Examiner has provided no objective evidence to refute this causal link. Accordingly, the Examiner has failed to make her *prima facie* case and the rejection of claims 18-32 under 35 U.S.C. §112, first paragraph, should be withdrawn.

Allowable subject matter.

Applicants note with appreciation that the Examiner has indicated that claim 1-32 are free of the art and that claims 1-14, 16, and 17 are allowed.

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

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Respectfully submitted,

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APPENDIX A

CLAIMS PENDING IN USSN 09/285,292 WITH ENTRY OF THIS AMENDMENT

1. A method of detecting a predisposition to cancer in an animal, said method comprising:

(i) providing a biological sample from said animal;

(ii) detecting the level of CYP24 within said biological sample; and

(iii) comparing said level of CYP24 with a level of CYP24 in a control sample taken from a normal, cancer-free tissue;

wherein an increased level of CYP24 in said biological sample compared to the level of CYP24 in said control sample indicates a predisposition to cancer in said animal.

2. The method of claim 1, wherein said level of CYP24 is detected by determining the copy number of CYP24 genes in the cells of said biological sample.

3. The method of claim 2, wherein said copy number is measured using Comparative Genomic Hybridization (CGH).

4. The method of claim 2, wherein said copy number is determined by hybridization to an array of nucleic acid probes.

5. The method of claim 3, wherein said Comparative Genomic Hybridization is performed on an array.

6 The method of claim 1, wherein said level of CYP24 is detected by measuring the level of CYP24 mRNA in said biological sample, wherein an increased level of CYP24 RNA in said sample compared to CYP24 RNA in said control sample indicates a predisposition to cancer.

7. The method of claim 6, wherein said level of CYP24 mRNA is measured in said biological sample and said control sample at the same vitamin D receptor activity or the CYP24 mRNA levels are normalized to the level of vitamin D receptor activity in the sample and control.

8. The method of claim 6, wherein said level of CYP24 mRNA is measured by hybridization to one or more probes on an array.

9. The method of claim 1, wherein said level of CYP24 is detected by measuring the level of CYP24 protein in said biological sample, wherein an increased level of CYP24 protein in said sample as compared to CYP24 protein in said control sample indicates a predisposition to cancer.

10. The method of claim 9, wherein the level of CYP24 protein is measured in the biological sample and the control sample at the same vitamin D receptor activity or the protein levels are normalized to the level of vitamin D receptor activity in the sample and control.

11. The method of claim 1, wherein said level of CYP24 is detected by measuring the level of 25-hydroxyvitamin D3 24-hydroxylase enzyme activity in said biological sample, wherein an increased level of 25-hydroxyvitamin D3 24-hydroxylase enzyme activity in said sample as compared to 25-hydroxyvitamin D3 24-hydroxylase enzyme activity in said control sample indicates a predisposition to cancer.

12. The method of claim 11, wherein said level of 25-hydroxyvitamin D3 24-hydroxylase activity is measured in said biological sample and said control sample at the same vitamin D receptor activity or the activity levels are normalized to the level of vitamin D receptor activity in the sample and control.

13. The method of claim 1, wherein said animal is a mammal selected from the group consisting of humans, non-human primates, canines, felines, murines, bovines, equines, porcines, and lagomorphs.

14. The method of claim 1, wherein said biological sample is selected from the group consisting of excised tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, and urine.

15. The method of claim 1, wherein the difference between said increased level of CYP24 in said biological sample and the level of CYP24 in said control sample is a statistically significant difference.

16. The method of claim 1, wherein said increased level of CYP24 in said biological sample is at least about 2-fold greater than the level of CYP24 in said control sample.

17. The method of claim 1, wherein said increased level of CYP24 in said biological sample is at least about 4-fold greater than said level of CYP24 in said control sample.

18. A method of estimating the survival expectancy of an animal with cancer, said method comprising:

(i) providing a biological sample from said animal;

(ii) detecting the level of CYP24 within said biological sample; and

(iii) comparing said level of CYP24 with the level of CYP24 in a control sample taken from a normal, cancer-free tissue;

wherein an increased level of CYP24 in said biological sample compared to the level of CYP24 in said control sample indicates a reduced survival expectancy in said animal compared to in an animal with cancer that has a normal level of CYP24.

19. The method of claim 18, wherein said level of CYP24 is detected by determining the copy number of CYP24 genes in the cells of said animal.

20. The method of claim 19, wherein said copy number is determined by hybridization to an array of nucleic acid probes.

21. The method of claim 19, wherein said copy number is measured using Comparative Genomic Hybridization.

22. The method of claim 21, wherein said Comparative Genomic Hybridization is performed on an array.

23. The method of claim 18, wherein said level of CYP24 is detected by measuring the level of CYP24 mRNA in said biological sample, wherein an increased level of CYP24 RNA in said sample as compared to CYP24 RNA in said control sample indicates a reduced survival expectancy.

24. The method of claim 23, wherein said level of CYP24 mRNA is measured in said biological sample and said control sample at the same vitamin D receptor activity or the activity levels are normalized to the level of vitamin D receptor activity in the sample and control.

25. The method of claim 18, wherein said level of CYP24 is detected by measuring the level of CYP24 protein in said biological sample, wherein an increased level of CYP24 protein in said sample as compared to CYP24 protein in said control sample, at a given level of vitamin D receptor activity indicates a reduced survival expectancy.

26. The method of claim 18, wherein said level of CYP24 is detected by measuring the level of 25-hydroxyvitamin D3 24-hydroxylase enzyme activity in said biological sample, wherein an increased level of 25-hydroxyvitamin D3 24-hydroxylase enzyme activity in said sample as compared to 25-hydroxyvitamin D3 24-hydroxylase enzyme activity in said control sample indicates a reduced survival expectancy.

27. The method of claim 26, wherein said level of 25-hydroxyvitamin D3 24-hydroxylase activity is measured in said biological sample and said control sample at the same vitamin D receptor activity or the activity levels are normalized to the level of vitamin D receptor activity in the sample and control.

28. The method of claim 18, wherein said animal is a mammal selected from the group consisting of humans, non-human primates, canines, felines, murines, bovines, equines, porcines, and lagomorphs.

29. The method of claim 18, wherein said biological sample is selected from the group consisting of excised tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, and urine.

30. The method of claim 18, wherein the difference between said increased level of CYP24 in said biological sample and the level of CYP24 in said control sample is a statistically significant difference.

31. The method of claim 18, wherein said increased level of CYP24 in said biological sample is at least about 2-fold greater than the level of CYP24 in said control sample.

32. The method of claim 18, wherein said increased level of CYP24 in said biological sample is at least about 4-fold greater than the level of CYP24 in said control sample.